



## Methods for the Isolation and Characterization of Constituents of Natural Products

### IV. Amide Derivatives of Amines with Pyruvyl Chloride 2,6-Dinitrophenylhydrazone

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In the first report of this series (2) preparation of a new acid chloride, pyruvyl chloride 2,6-dinitrophenylhydrazone and esters resulting from the condensation of it with primary, secondary, and tertiary aliphatic alcohols was described. The potential utility of the reagent and resultant derivatives was discussed from the standpoint of the ease with which the derivatives can be formed in and fractionated from a complex lipid mixture, and their subsequent isolation free of contamination. As a continuation of a program to develop new methods for the isolation and characterization of functional constituents in natural products, the present paper describes the preparation of a homologous series of the pyruvic acid 2,6-dinitrophenylhydrazone derivatives of primary amines and a partial series of secondary amine derivatives. These derivatives exhibit properties much like their ester analogues not only in their physical characteristics but also in the ease with which they are formed and isolated, especially at the micromole or submicromole level. These characteristics should make them particularly useful in the analyses of natural products.

#### APPARATUS AND REAGENTS<sup>1</sup>

The apparatus and reagents used to prepare the amides were identical to those described for the preparation of the esters (2).

All primary amines were straight-chain. All secondary amines were

<sup>1</sup> Reference to certain products or companies does not imply an endorsement by the Department over others not mentioned.

straight-chain and symmetrical. All amines were purchased from Lachat Chemical Inc., Chicago, Illinois, except for the following: methyl-, ethyl-, and dimethylamine were purchased as compressed gases from the J. T. Baker Company, Phillipsburg, New Jersey; diethylamine and di-*n*-propylamine were obtained from the Matheson Company, East Rutherford, New Jersey. All of the amines were of the highest purity available from the specified sources and were used without further purification.

#### EXPERIMENTAL

*Amides of pyruvic acid 2,6-dinitrophenylhydrazone.* The procedure for the preparation of the amides differed slightly from the preparation of esters (2). A stock solution of pyruvyl chloride 2,6-dinitrophenylhydrazone is made by dissolving 23.5 mg (0.08 mmole) of the acid chloride per milliliter of purified benzene. Ten milliliters of this solution are pipetted into a dry flask and the amine is added. Four or five drops of *n*-propyl through *n*-hexyl, diethyl- and di-*n*-propylamines are used. For longer-chain amines, 0.4 mmole is weighed out and transferred to the flask using a minimum of purified benzene to effect transfer when necessary. Five milliliters of a solution of triethylenediamine (containing 19.4 mg/ml of purified benzene) are added in 1-ml portions, shaking briefly between additions and the mixture allowed to stand for 5 minutes or more at room temperature. For preparation of the derivatives of methyl-, ethyl-, and dimethylamine, the gas is bubbled briefly through 10 ml of the acid chloride solution until an orange-red color is observed. Triethylenediamine is not added to these solutions.

The amide derivatives are isolated on weakened, acidic alumina in the same manner as was described for the esters (2) except that the secondary amine and short-chain primary amine derivatives are eluted from the column with chloroform. A green color may develop on the alumina in the case of methyl-, ethyl-, and dimethylamine derivatives. This is due to the presence of a large excess of amine. No adverse effect is evident from this, however, and the excess base is removed in the evaporation step.

For all of the derivatives, the solvent is removed on the steam bath under a gentle stream of air or nitrogen, and the residue is recrystallized from absolute ethanol. The yield is approximately 90%. The amides were recrystallized to a constant molar extinction coefficient. This was found to be a more reliable index of purity for the individual compounds than was a constant melting point. Most of the primary amines gave an

analytically pure sample after one recrystallization. The shorter-chain amines and most of the secondary amines required two recrystallizations.

# RESULTS AND DISCUSSION

The physical constants determined on the amides are given in Tables 1 and 2. All of the derivatives are solid, highly crystalline materials that melt sharply without decomposition. Primary amine derivatives are yellow, and exhibit an absorption maximum at 407 m $\mu$  in benzene; derivatives of secondary amines are orange with an absorption maximum in benzene near 412 m $\mu$ . Both classes of derivatives color more or less violet in alcoholic base, a phenomenon displayed by many nitro- and polynitro compounds (1). This feature is associated with chemical changes which facilitate their subsequent isolation from a natural product since it enables them to adsorb strongly onto alkaline adsorbents, to ion-exchange under the proper conditions, and to extract into alkaline solution. Schwartz

TABLE 1  
PHYSICAL PROPERTIES OF AMIDES OF PRIMARY AMINES WITH PYRUVIC ACID  
2,6-DINITROPHENYLHYDRAZONE

Pri- mary amines	M.P. <sup>a</sup> (°C)	E <sup>b</sup>	C (calc)	C (found)	H (calc)	H (found)
C <sub>1</sub>	208 — 209	5788	42.7	42.9	3.91	4.41
C <sub>2</sub>	165.5 — 167	6008	44.7	45.2	4.41	4.84
C <sub>3</sub>	127 — 128	5907	46.6	46.7	4.85	5.00
C <sub>4</sub>	76 — 77	5924	48.3	48.1	5.26	5.18
C <sub>5</sub>	58 — 59	5976	49.8	49.8	5.63	6.31
C <sub>6</sub>	77 — 78	6038	51.3	50.9	5.98	6.04
C <sub>7</sub>	67	5953	52.6	52.6	6.31	6.06
C <sub>8</sub>	55	5963	53.8	54.8	6.59	6.74
C <sub>9</sub>	59 — 60	5976	55.0	55.3	6.88	7.19
C <sub>10</sub>	66 — 66.5	5986	55.9	55.9	7.12	7.13
C <sub>11</sub>	67.5 — 68	5919	57.0	57.8	7.37	7.52
C <sub>12</sub>	77	5902	57.9	57.8	7.59	7.76
C <sub>13</sub>	74 — 74.5	5996	58.8	58.0	7.78	5.04
C <sub>14</sub>	78 — 78.5	6059	59.7	59.4	7.96	7.88
C <sub>15</sub>	78 — 79	6162	60.3	60.3	8.17	8.29
C <sub>16</sub>	85.5 — 86	6136	61.0	61.0	8.35	8.44
C <sub>17</sub>	82.5 — 83	6094	61.8	60.1	8.51	8.64
C <sub>18</sub>	89.5 — 90.5	5938	62.5	63.0	8.67	8.92
Average		5941				

<sup>a</sup> Determined with the Fisher-Jones apparatus and uncorrected.

<sup>b</sup> Determined in benzene in a 1-cm<sup>2</sup> cell at 407 m $\mu$ .

TABLE 2  
PHYSICAL PROPERTIES OF AMIDES OF SYMMETRICAL SECONDARY AMINES WITH  
PYRUVIC ACID 2,6-DINITROPHENYLHYDRAZONE

Sec- ondary amines	M.P. <sup>a</sup> (°C)	E <sup>b</sup>	C (calc)	C (found)	H (calc)	H (found)
C <sub>2</sub>	133.5 — 136	5323	44.7	45.8	4.40	4.63
C <sub>4</sub>	115.5 — 117	5408	48.3	48.1	5.88	5.39
C <sub>6</sub>	150 — 151	5368	51.3	51.2	6.55	6.33
C <sub>8</sub>	93 — 93.5	5403				
C <sub>10</sub>	46 — 48	5335	56.0	56.1	7.61	7.37
	Average	5367				

<sup>a</sup> Determined with the Fisher-Jones apparatus and uncorrected.

<sup>b</sup> Determined in benzene in a 1-cm<sup>2</sup> cell at 412 mμ.

*et al.* (3) have utilized these properties in 2,4-dinitrophenylhydrazones to isolate and fractionate the derivatives formed directly in lipids.

Preliminary work indicates that both primary and secondary amines can be derivatized at the submicromole level under very mild conditions. The quantitative aspects of this as well as the separation of primary and secondary derivatives into classes and subsequent resolution of the individual members is also being investigated.

#### SUMMARY

Preparation of amide derivatives of primary and secondary aliphatic amines with pyruvyl chloride 2,6-dinitrophenylhydrazone is described. The derivatives form within 5 minutes at room temperature in benzene solution. Primary amine derivatives are yellow, and all members investigated (normal C<sub>1</sub>-C<sub>18</sub>) are solids. The primary amine derivatives have an absorption maximum at 407 mμ in benzene and have a molar absorptivity near 5941. Secondary amine derivatives are orange, and all members investigated (symmetrical, unbranched C<sub>2</sub>-C<sub>10</sub>) are also solids. The secondary amine derivatives have an absorption maximum at 412 mμ in benzene and have a molar absorptivity near 5367. Both classes of amine derivatives have properties which facilitate their isolation directly from lipids.

#### REFERENCES

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